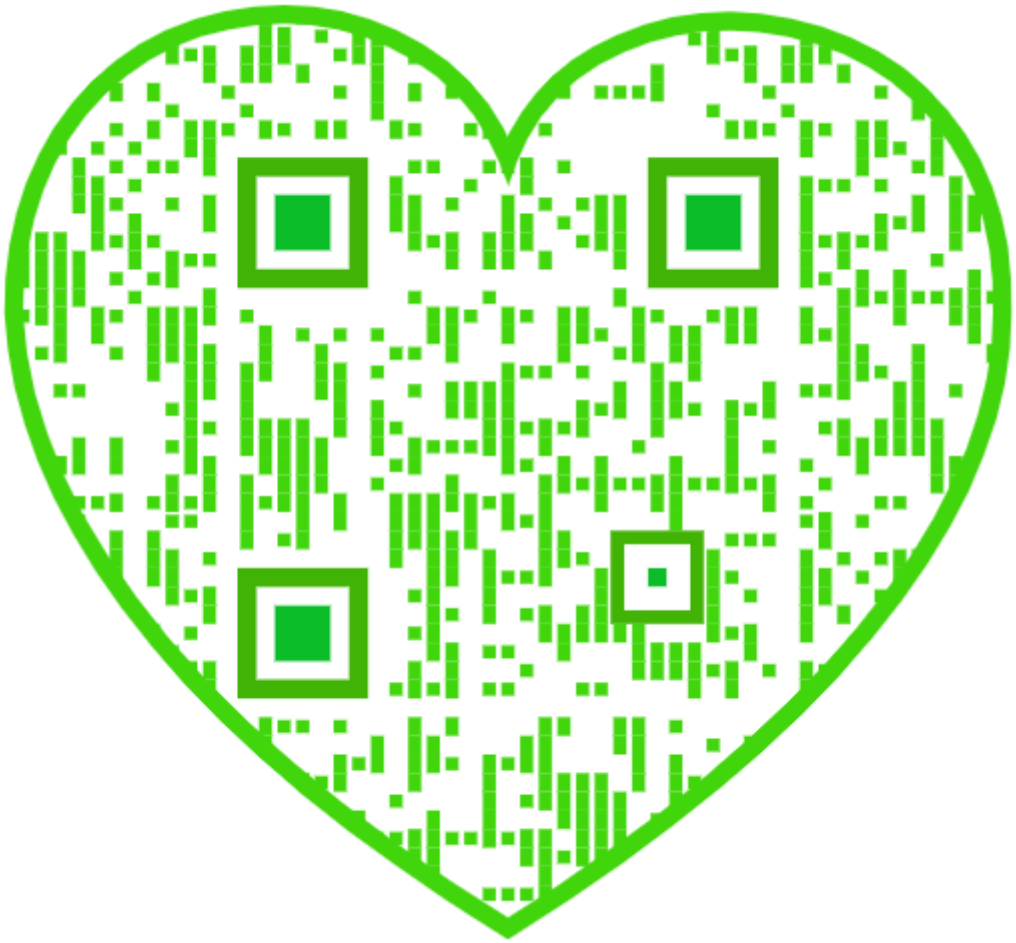

BULLETIN OF THE
U.S. DEPARTMENT OF AGRICULTURE

No. 127

Contribution from the Bureau of Plant Industry, Wm. A. Taylor, Chief.
September 16, 1914.

(PROFESSIONAL PAPER.)

THE MYCOGONE DISEASE OF MUSHROOMS AND ITS CONTROL.

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INTRODUCTION.

The industry of mushroom growing in this country has been steadily increasing until to-day large establishments for the cultivation of mushrooms are found in the vicinity of nearly all of the large cities. In the eastern part of Pennsylvania there are many extensive mushroom plants which supply the eastern markets. In one section there are more than 250 establishments whose collective product exceeds 1,000,000 pounds of mushrooms annually, while many of the growers individually send to market over 100,000 pounds a year. The substantial manner in which the modern mushroom houses are constructed and the extent and operation of the individual plants represent investments of considerable magnitude; consequently, the failure of a crop in even one mushroom house means a serious financial loss to the grower. Because the knowledge and conditions necessary for the successful cultivation of mushrooms are peculiar and unique, and while it is recognized that various factors—such as an unsuitable degree of humidity, imperfect ventilation, improper preparation of the beds, the presence of insects, and other unfavorable conditions—may be the cause of the loss of a crop or a large percentage of it, the growers have only recently been led to appreciate that a fungous disease is responsible for extensive losses.

PREVALENCE OF THE DISEASE IN MUSHROOM BEDS.

Many instances of total failures of mushroom houses are recorded. For example, one grower reports the complete failure of four houses and about two-thirds of another house. Houses that should have

Note.—This bulletin describes a disease of mushrooms which causes great losses to growers and gives methods of control. Of interest to mushroom growers generally.

produced more than 30,000 pounds yielded less than 1,000 pounds of mushrooms, owing to the ravages of this fungous disease. An establishment containing over 50,000 square feet of mushroom beds was abandoned on account of the heavy losses sustained.

While the disease is prevalent in most localities where mushrooms are cultivated on a large scale, there are mushroom-raising plants in these infected districts where the disease has never made its appearance. There is, however, evidence of a local distribution of the disease, and unless proper measures to control the fungus are taken it will be only a question of time before all mushroom houses are infected.

Costantin and Dufour (1892*c*)[1] state that under the ordinary conditions of cultivation in a great number of the caves or underground quarries in which mushrooms are cultivated abroad, the proportion of diseased to normal mushrooms is about 1 to 10. In less frequent cases this proportion rises to 1 to 4. Costantin and Dufour and Répin (1897) report the losses to the Parisian mushroom growers caused by this disease to be about $200,000 yearly. This loss is based on a daily estimated production of about 56,000 pounds, at 66 cents per pound, the yearly production having a value of approximately $2,600,000 and losses due to the disease being estimated at probably one-tenth of the production. As nearly as can be ascertained these figures apply to the year 1892.

OCCURRENCE OF THE DISEASE IN AMERICA.

The occurrence of the fungous disease of cultivated mushrooms in America was first called to the attention of the Department of Agriculture in 1909, when specimens of diseased mushrooms were sent to the department with requests for a diagnosis of the trouble. A microscopic examination revealed the presence of a fungus, a species of Mycogone, similar to, if not identical with, the species causing the European disease of mushrooms known as la môle.

HISTORICAL REVIEW OF THE MYCOGONE DISEASE.

OCCURRENCE IN EUROPE.

The Mycogone disease of cultivated mushrooms has been known in England, France, and Germany for many years. In France it is reported as having been recognized "for at least three generations of mushroom growers," and it is believed that it was known at a much earlier date. Probably the first reference to the disease in scientific literature was that of Magnus, who described it in 1888 as being the most serious enemy of mushroom growing around Berlin.

[1] All references to literature are indicated in the text by the name of the author and the year of publication. For full citations, see the list at the end of this bulletin.

IDENTITY OF THE FUNGUS.

The common identity of the fungi studied by different investigators in relation to the disease of mushrooms has not been established, but it is interesting to note that in each study one of the three concerned in the life history of the fungus causing the mushroom disease, e. g., Mycogone, Verticillium, or Hypomyces, is the subject of investigation. Owing to the similarity of the fungus causing the disease to certain stages of diseases of wild species of mushrooms, Magnus called the fungus *Hypomyces perniciosus*. No technical description was given by this author and the fungus was only a hypothetical stage of Hypomyces.

Cooke (1889) in England identified the fungus which was causing considerable loss to mushroom growers as "a species of Mycogone not unlike *Mycogone rosea* in many of its features, but referable to *Mycogone alba*," notwithstanding the fact that he described the larger cells of the Mycogone spores as becoming amber colored.

In Vienna, Austria, Stapf (1889) described a disease of cultivated mushrooms, which was attributed to *Verticillium agaricinum* Corda, a conidial stage of *Hypomyces ochraceus* Pers. Stapf found several spores of Mycogone, but could not connect them with Verticillium.

Prillieux (1892) described a disease of mushrooms and identified it as *Mycogone rosea*, which by analogy he considered a conidial stage of *Hypomyces linkii* Tulasne, although the perfect stage of the fungus had never been observed.

In the same year Costantin and Dufour (1892*a*) published a note on the disease then known as "la molle." They described the macroscopic and microscopic characters of the disease and stated that the fungus is very similar to *Mycogone cervina*, though it differs in habitat, or host plant. Two types of modification of the mushroom caused by the fungus are described. In the first type the cap, stipe, and gills are well defined, though the presence of the disease is indicated by a stipe swollen at the base, swelling of the gills, and distortion of the cap. The second type of the disease is manifested by an early arresting of the development of the mushroom, the cap is rudimentary or entirely lacking, and the stipe, or stem, is swollen to such a size that the affected mushroom has the appearance of a puffball. On the diseased mushrooms of the first type Mycogone and Verticillium spores were found. The Verticillium spores were long, cylindrical-oblong, and sometimes two celled. The relationship of these two forms, Mycogone and Verticillium, was established by finding the two kinds of spores growing on the same mycelium. The second or puffball-like type of diseased mushrooms has only a Verticillium with small unicellular spores, microscopically quite different from the Verticillium of the first type.

It was at first thought that there were two different diseases, but when a sufficient number of specimens were studied all transitions between the two forms of Verticillium were found and it was established that it was one disease with two very dissimilar forms of fruiting bodies.

Costantin explained that, together with Dufour, he had previously stated that the fungus was similar to *Mycogone cervina*, but that they had not identified it with that species. The fungus also did not agree with the description of *Mycogone rosea*.

Costantin and Dufour (1892*c* and 1893*a*) give the most exhaustive study of this disease of cultivated mushrooms. They discuss the two characteristic types of infection, as described in their previous article, designating them as the common form and the scleroderma or puffball-like form. The discussion of the fungus immediately following embodies the observations made by these authors. A microscopic examination of the common form, as previously described, revealed the presence of a Verticillium having large spores variable in size and form. The large spores are two celled, 16 to 20 by 3.5 μ, while the small spores are more numerous and one celled, 8 by 3 μ. This Verticillium is usually found on the gills in an early stage of the disease. At a later stage of the disease the mushroom is covered with a thick white coating consisting of long irregularly and verticillately branched hyphæ, upon which are borne bicellular "chlamydospores" of a Mycogone. In rare cases these large Mycogone spores are three celled and much longer than the usual 2-celled spores. At first these two cells are smooth, hyaline, or colorless, and almost equal in size; later the terminal cell becomes swollen, amber colored, and covered with warts. It is spherical in shape and measures about 16 to 20 μ. The lower or basal cell is smaller, smooth, colorless, and 14 to 16 μ in size.

In specimens of the puffball-like type of infection the color is at first a dirty white, becoming pearl gray or pale rose gray as the disease advances. In this latter stage the deformed mushroom is covered with a light velvety tomentum or hairlike coating formed of little tufts or filaments much more branched and scattered and thinner, but still of the form of Verticillium. The spores are unicellular, more numerous than the form just described, and much smaller, being 4 by 2 μ in size.

Magnus (1906), Cooke (1889), and Prillieux (1892) probably observed the disease in the common form, i. e., the Verticillium with large spores and the Mycogone spores, while Stapf (1889), who reported only a Verticillium stage, examined the disease in the puffball-like stage, where only the Verticillium with small spores was present.

As previously suggested by Costantin and Dufour, it might be supposed that these two forms of Verticillium belonged to two distinct diseases. A specimen was found infected in the puffball-like manner which had a rose-gray velvety covering and at the same time a whitish woolly coating, on the lower part the Verticillium with small spores, and on the upper part Mycogone and the Verticillium with large spores. There was a gradual transition from the Mycogone and large-spored Verticillium to the small-spored Verticillium. The conclusion is then drawn that there is one disease with two forms of Verticillium. This is an interesting fact, since Costantin and Dufour by sowing the large-spored Verticillium in suitable media produced Mycogone spores. The cultures were at first drab in the center and white at the margin, finally becoming a color intermediate between light leather and umber. It was impossible to cultivate the large-spored Verticillium alone, as this form was always accompanied by the production of Mycogone spores.

When cultures were made from the small-spored Verticillium of puffball-like infected mushrooms, they remained permanently white and only the Verticillium with small spores was produced. Never could the small-spored Verticillium be made to take the characters of the large-spored Verticillium.

It is concluded that this parasite of the cultivated mushrooms differs from *Mycogone rosea* and *Mycogone cervina* in habitat, size, and color of the "chlamydospores," but that it is the species named by Magnus (1906) *Hypomyces perniciosus*, although his description was insufficient and he regarded the "chlamydospores" as being hyaline, whereas this is only the case in immature spores, for they rapidly become amber colored. The authors designate the species as *Mycogone perniciosa* Magnus.

Evidently Prillieux (1892), who believed the species to be *Mycogone rosea*, later agreed with Costantin and Dufour in considering it *Mycogone perniciosa*, for the sketch he presented before the Botanical Society of France has been reproduced and designated as *Mycogone perniciosa* (Prillieux, 1897).

Delacroix (1900) described the disease "la môle" as the most important of the diseases of cultivated mushrooms, and ascribed it to the fungus *Mycogone perniciosa* Magnus.

Magnus (1906) discussed the work of preceding investigators and concluded that the *Verticillium agaricinum* (Lk.) Cda. given by Stapf (1889) as causing a serious disease of cultivated mushrooms at Vienna is rather *Mycogone rosea* Lk. than *Mycogone perniciosa* Magnus as given by Costantin and Dufour. However, the question of specific identification is one which Magnus states requires further study.

The fungus causing the disease in American houses is probably the one described by Costantin and Dufour (1892*c*) and called *Mycogone perniciosa* Magnus. Costantin (1893*a*) gives the morphological differences between *Mycogone rosea* and *Mycogone perniciosa*, and, although there has been no opportunity to compare our fungus with the European species, it is thought that the two species are identical, as they agree in both macroscopical and microscopical characters.

INVESTIGATIONS OF THE MUSHROOM DISEASE IN AMERICA.

TYPES OF THE DISEASE.

During the course of the present investigation, specimens of the two types or forms of infection as described by the French investigators were collected in diseased mushroom houses in this country. In many cases it was difficult to determine to which type the diseased individuals belonged, as there are gradations between the two forms. Often cap, stipe, and gills are clearly defined, the presence of the malady being indicated by small tubercles on the cap and a fluffy white growth on the gills, a form of the disease known in France (Costantin and Dufour, 1892*c*, p. 471) as "chancre."

In this country the common form of the disease is similar to that in France. The mushrooms are covered with a white, velvety coating, which consists of interwoven hyphæ. This growth prevents the normal development of the individual gills, which become more or less coalescent, a condition shown in Plate I. The progress of the disease is also frequently accompanied by arrested development and by the distortion of the cap and stipe, as well as by the general darkening and decay of the tissue. These characters are illustrated in Plates II and III.

In cases of infection termed by the French the "scleroderma" form, the stipe is bulbous and the cap rudimentary or entirely lacking. In this form the gills are completely aborted, and the diseased mushroom is covered with a coating of interwoven hyphæ similar to that of the common form. It has been observed that in this form of the disease the plants are much softer than in the other form and that they decay more rapidly. Monstrous soft masses with thick white coatings of the fungus are often observed in houses in which the disease is abundant. These infected plants have very little resemblance to mushrooms, and they decay rapidly, forming a putrid mass which emits a disagreeable, almost acrid odor. Figure 1 illustrates one of these masses. Clumps greatly exceeding this in size are often found.

MICROSCOPIC CHARACTERS OF THE FUNGUS.

The small-spored Verticillium described by Costantin and Dufour (1892*c*) has not been observed in the specimens examined from American houses, but it has been possible to grow the Mycogone in

THE COMMON OR USUAL TYPE OF MALFORMATION OF MUSHROOMS, ILLUSTRATING THE DESTRUCTIVE EFFECT OF THE MYCOGONE DISEASE.

These specimens are covered with the cottony growth of the parasite, two of them showing the coalescence of the gills.

DISEASED MUSHROOMS, ILLUSTRATING MALFORMATIONS DUE TO THE MYCOGONE DISEASE.

The cross section shows the darkening or decay of the tissue from the same cause.

EXAMPLES OF THE PUFFBALL-LIKE TYPE OF DISEASED MUSHROOMS AND TWO NORMAL SPECIMENS.
The characteristic distortion of mushrooms of this type is clearly shown in comparison with the healthy specimens.

cultures from inoculations made indiscriminately from the common and sclerodermalike forms. Further investigation, however, may demonstrate the occurrence of this small conidial form.

Figure 2 illustrates the Mycogone stage of the disease. During the course of the present investigation many hundreds of cultures were made, both from infected material and by transfers from pure cultures of the fungus. With few exceptions, spores of Verticillium developed first and later were followed by Mycogone. The spores of Verticillium are hyaline, oblong cylindrical, and borne on tapering branches. They are generally one celled, but occasionally larger, 2-celled spores are developed. They are variable in size, the average measurement being 20 by 3.5 μ for the larger 2-celled spores. The cell wall is uniformly thin. Often, as noted by Costantin and Dufour

FIG. 1.—Irregular mass of a diseased mushroom growing among normal mushrooms.

(1892*c*) and Prillieux (1897), these Verticillium spores were borne on the same hyphæ as the Mycogone spores. The Mycogone spores were usually produced at the bases of the hyphæ strands and the Verticillium spores at the apexes.

Figure 2 is an illustration of the Mycogone spores and the manner in which they are produced on the mycelium. They are two celled, the upper cell spherical and rough or covered with warts. At first, both cells are hyaline or colorless; later, the upper cell becomes light brown, the lower cell usually remaining hyaline, but in rare cases becoming faintly tinged with brown, averaging in size from 20 to 30 μ. The cell walls are thick, while the spores of the Verticillium possess very thin walls.

THE DISEASE IN MUSHROOM BEDS.

The study of the mushroom disease in America has proved the fungus to be exceedingly variable as regards the time of its appearance. In some instances evidences of Mycogone were observed when the mushrooms were just beginning to appear; again, the crop was well developed before being attacked by the parasite. The experience of the French growers also proves that the time of the appearance of the disease is subject to great variation, but that ordinarily it reaches its height about the middle of the productive period of the beds.

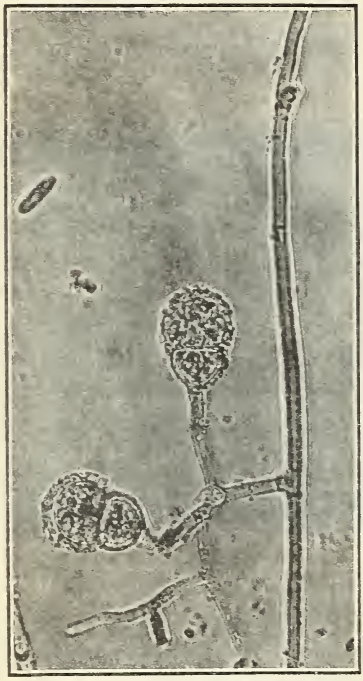

FIG. 2.—Mycogone spores, showing the thick-walled and warty cells. Magnified 425 diameters. From a photomicrograph by Dr. Albert Mann.

The occurrence of diseased mushrooms in an infected house is very sporadic. Sometimes isolated diseased specimens will appear among normal mushrooms, while again perfectly healthy mushrooms will be observed growing in a badly diseased bed. An example of a badly diseased specimen growing among normal mushrooms is illustrated by figure 1.

PROPAGATION AND DISSEMINATION OF THE DISEASE.

The manner in which the parasite is propagated in the beds is only partially known, but from the experience of foreign growers and the studies of the writer there can be little doubt that the disease is distributed by the spawn and that the fungus grows up with the mycelium of the mushroom, which it finally attacks and destroys.

A thorough investigation of one of the American mushroom spawn-manufacturing plants in which the so-called "tissue method" of spawn making is practiced leads to the conclusion that there is little chance of the disease being carried by the spawn where proper precautions are taken to prevent infection. To prevent infection of the spawn entails great care on the part of the manufacturer. He must be absolutely sure that his cultures are made under sterile conditions and that the bricks are kept from any chance of contamination by spores of the parasite. Investigation and inquiry among most of the large growers in this country have dis-

closed the fact that the disease is as prevalent in the beds of growers who use imported spawn entirely as among those who use domestic spawn. Instances of the use of both foreign and American manufactured spawn by growers in localities where the disease was present have been noted in which there was no trace of the disease. It is the general opinion among American growers that the disease was introduced by imported spawn.

In the course of the present investigations it has been possible to propagate the parasite in the laboratory on pieces of blank spawn bricks [1] in sterilized bottles. The mycelium of the fungus spreads over the pieces of brick, eventually fruiting and producing spores of Verticillium and Mycogone. These experiments prove that under proper conditions the parasite will grow on the spawn bricks. The growth of the fungus appeared to be superficial, and it could not be ascertained whether or not the mycelium penetrated the bricks. No reliable method has yet been evolved to determine the presence or absence of the mycelium of Mycogone in spawn bricks. The observation of the writer has been that there are no marked differences between spawn in an infected bed and that in healthy beds.

Although our knowledge is incomplete as to the exact way in which the parasite spreads through the beds, because of insufficient experiments on this phase of the subject, it seems probable from the limited data that the parasite does grow through the manure of the mushroom beds and attacks the developing mushrooms, producing spores by means of which the disease may be carried to other beds. In addition to this method of reinfection, the question suggests itself as to whether the fungus may not persist for long periods in the lumber used in the construction of houses or beds. In order to determine this point many cultures were made from the wood secured from diseased houses, but at the present time no definite conclusions can be drawn.

LONGEVITY OF THE FUNGUS.

In order to obtain data which would be of assistance in devising a method for the control of the mushroom disease, two distinct lines of investigation on the subject of the longevity of the fungus were inaugurated. Laboratory and field experiments were continued during a period of over three years. While the experiments were not sufficiently exhaustive to be conclusive, they are significant and interesting.

Laboratory experiments.—Many different sets of cultures were made on corn meal in 100-cubic-centimeter flasks. These were opened, examined, and transferred at certain periods in order to ascertain

[1] Blank spawn bricks are bricks in which the mushroom mycelium has not been "run," or grown.

their power of germination. Several hundred cultures were made during this series of experiments, and no growth was produced from cultures after a period of 18 months. It should be stated, however, that the cultures were kept in a dry place and were consequently thoroughly dried out at the termination of 18 months. That the organism retains its vitality for such a period and on an artificial medium demonstrates its dangerous nature. The probability is strong that in the mushroom house, under more favorable conditions of humidity and temperature, it would retain its power of germination much longer.

Field observations.—The writer's opportunity to study the vitality of Mycogone in the beds of different mushroom houses has covered a period of about three years. One case for observation was that of a new house in which earth was mixed with manure from a badly infected house, which manure had been exposed to the weather for five years. This house produced a splendid crop of mushrooms, which indicates that the fungus exposed to the weather had not retained its vitality at the close of this period. A case in which the fungus persisted for three years is cited by Costantin. Two caves were held under observation; one was new, while the second had been used for mushroom cultivation for over 30 years, although the culture had not been continuous. This second cave was idle for three years before being employed for this experiment. The yield of the new cave exceeded that of the second, and the presence of the disease in the first was negligible, while in the second it was considerable. The conditions and attention in the two caves were practically the same, and, while the report does not mention what precautions were observed to prevent infection of the new beds, the result would indicate that the spores in the abandoned cave had retained their vitality for at least three years.

CULTURAL STUDIES TO DETERMINE A MEANS OF CONTROL.

Many hundreds of cultures of Mycogone were made during the course of the present investigation, for the purpose of studying the development and habit of the fungus and observing the direct effect of fungicides and disinfectants upon the organism. It was found that the fungus could be cultivated upon numerous different media, but as corn meal in flasks and corn-meal agar proved very congenial media they were employed in most of the experiments. The fungus grew rapidly, producing vigorous cultures, and practically no difficulty was experienced from contaminations even when fresh cultures were made directly from diseased mushrooms on which spores of various fungi were doubtless present. Figure 3 shows a photograph of one of the cultures, all of which exhibited a similar vigorous growth. In the early stages the cultures of the fungus were white, soon becom-

ing drab in the center and finally light brown. As the method of growth is centrifugal and the marginal growth of the culture is the youngest, it is the last to become brown. The most important physical factor concerned in the growth of the fungus in culture proved to be humidity. While ordinarily low temperatures are not conducive to the growth of fungi, in the present instance it was found that a moist atmosphere was more important to the growth of the cultures than a high temperature. This observation was made from the study of numerous cultures in flasks and tubes and on various media subjected to different degrees of humidity maintained at various temperatures. Cultures grew at a temperature as low as 2° C. (35.6° F.) when considerable moisture was present, while cultures at a temperature of 35° C. (95° F.) in a dry atmosphere failed to produce any growth. This peculiarity of the fungus is an important factor in the method of fumigation.

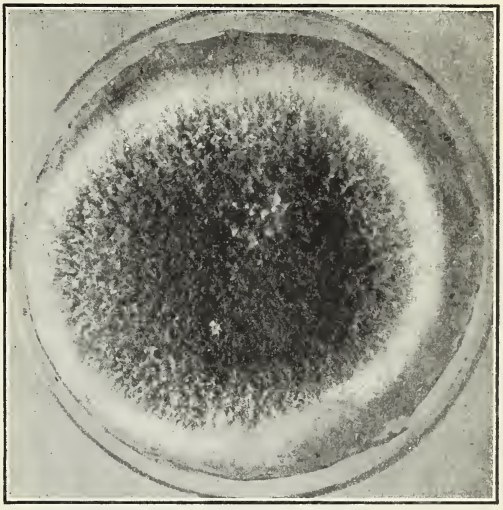

FIG. 3.—Petri-dish culture of Mycogone on corn meal, 21½ hours after inoculation made from an infected mushroom.

TREATMENT WITH FORMALDEHYDE GAS.

In view of the important rôle of formaldehyde as a disinfectant and fungicide and the success obtained from its use in inhibiting the growth of certain parasitic fungi (Patterson, Charles, and Veihmeyer, 1910), a series of laboratory experiments was performed to determine the effect of formaldehyde upon the Mycogone disease of cultivated mushrooms.

The apparatus used in these experiments is fully described and illustrated in Bulletin No. 171 of the Bureau of Plant Industry. It consists of a large air-tight box provided with a glass door and a set of drawers, whereby cultures can be withdrawn from the box at any time during the process of fumigation with a minimum loss of the formaldehyde gas.

CHEMICALS EMPLOYED.

The formalin employed in these experiments was purchased in the open market and was supposed to be of full strength, which should contain 40 per cent by volume of formaldehyde gas (37 per cent

weight U. S. P. standard). The formalin-permanganate method was used to generate the gas in all the experiments, since it has been found that it is the only practicable way of using the gas in mushroom houses. By this method formalin is poured on crystals of potassium permanganate, and this was the procedure in the laboratory experiments, but in the practical application of the method it was found necessary to deposit the potassium permanganate in the receptacles containing the formalin. Chemically pure potassium permanganate in finely divided crystals was used. In each of the experiments the proportions were 100 cubic centimeters of formalin to 50 grams of potassium permanganate.

Exposures of Cultures.

In the laboratory experiments, pure cultures of the fungus were subjected to the direct action of the formaldehyde gas. The cultures were exposed to the gas in Petri dishes and 100-cubic-centimeter flasks.[1]

Fig. 4.—Check culture, experiment 5: Transfer made from culture before it was subjected to formaldehyde gas. From a photograph made 189½ hours after inoculation.

Transfers for checks were made from the cultures to be subjected to the action of the gas immediately before the fumigation and again immediately after fumigation, in order to determine the effect of the formaldehyde gas on the vitality of the fungus. Figure 4 shows the manner in which the inoculations were made in every case in Petri dishes. There were four centers of inoculation, and the amounts transferred from the fumigated cultures were large enough to allow abundant chance for the fungus to grow. In the flask cultures four inoculations were also made.

Results of Fumigation with Formaldehyde on Cultures of the Fungus.

The results of these experiments are given in Table I. The efficacy of formaldehyde in destroying the parasite is clearly shown by these experiments. As stated by McClintic (1906), and also by Patterson, Charles, and Veihmeyer (1910), it seems that higher temperature and humidity increase the fungicidal action of the gas.

[1] The cultures contained Mycogone and Verticillium, as in no instance was it possible to cultivate the Verticillium alone.

TABLE I.—*Results of experiments with formaldehyde gas on cultures of the fungus.*

Experiment.		Temperature (°F.) before mixing reagents.	Formalin (c. c.) per 1,000 cubic feet of space.	Age (in days) of culture at time of fumigation.	Number of cultures exposed to formaldehyde gas for—		Number of check cultures made.	Growth (period in days)[1] of the fungus in checks and in transfers made from cultures exposed to the gas for 30 and 60 minutes, respectively.		
Date.	No.				30 minutes.	60 minutes.		Check.	30 minutes.	60 minutes.
1911.										
Jan. 31	1a	55	700	21	12	13	4	3¾	3 in 6; 1 in 8; 2 in 18	1 in 8; 1 in 21.
	1b	55	700	17	11	11	4	3¾	5 in 6; 3 in 7	2 in 6; 2 in 8.
	1c	55	700	7	11	12	4	3¾	6 in 6; 1 in 7; 1 in 14; 1 in 17; 1 in 26	1 in 7; 1 in 21; 1 in 26.
Feb. 8	2	67	700	11	22	23	8	3	1 in 8½; 1 in 20; 1 in 25; 1 in 28	3 in 4½; 2 in 5½; 1 in 9½; 1 in 28.
Mar. 1	3	65	800	16	19	18	8	3	No growth	1 in 5½.
Mar. 10	[2] 4	76	900	23	16	18	10	3½	do	No growth.
Mar. 16	5	58	1,000	12	14	15	13	3½	do	Do.
Apr. 4	[3] 6	74	900	17	None.	12	4	4		1 in 38.
	[3] 7	76	800	9	11	11	6	3½	No growth	No growth.
Apr. 14	[4] 8	78	700	11	10	9	5	[5] 4 in 3½	do	Do.
Apr. 20	[6] 9	73	600	13	12	10	6	5 in 3½	1 in 8½; 1 in 26	1 in 8½; 1 in 17; 1 in 20.
Apr. 25	[7] 10	76	500	20	None.	15	6	5 in 3½; 1 in 4½		1 in 10; 1 in 23.

RECAPITULATION OF RESULTS.

Experiment No.	Temperature (°F.) before mixing.	Formalin (c. c.) per 1,000 cubic feet of space.	Age (in days) of culture at time of fumigation.	Percentage of check cultures and transfers from cultures which grew after exposure to formaldehyde gas for stated lengths of time.					
				Checks.	15 minutes.	30 minutes.	60 minutes.	90 minutes.	18½ hours.
10	76	500	20	83.33	42.85	.....	13.33	.....	0
9	73	600	13	83.33	.....	16.67	30	0	0
8	78	700	11	80	0	0	0	0	.....
1a	55	700	21	100	.....	58.33	15.38	.....	.....
1b	55	700	17	100	.....	72.72	36.36	.....	.....
1c	55	700	7	100	.....	90.90	25	.....	.....
2	67	700	11	100	.....	18.18	34.35	.....	.....
3	65	800	16	100	.....	0	5.55	.....	.....
7	76	800	9	100	0	0	0	0	.....
4	76	900	23	100	.....	0	0	0	.....
6	74	900	17	100	.....	.....	8.33	.....	.....
5	58	1,000	12	100	.....	0	0	.....	.....

[1] The period for growth was reckoned to the time when an indication of growth of the fungus in the transfers first appeared.

[2] In experiment 4, 17 cultures also were subjected for 90 minutes to formaldehyde gas, and the transfers from them showed no growth.

[3] In experiment 7, 12 cultures were also subjected for 15 minutes and 11 cultures for 90 minutes to formaldehyde gas, and the transfers from them showed no growth.

[4] In experiment 8, 10 cultures were also subjected for 15 minutes to the gas and 10 cultures for 90 minutes. None of the transfers grew.

[5] One culture contaminated.

[6] In experiment 9, 12 cultures were also subjected for 90 minutes and 11 cultures for 18½ hours to the gas. There was no growth in the transfers.

[7] In experiment 10, 14 cultures were also subjected for 15 minutes to the gas, and 6 transfers grew in 16 days; 14 cultures also were subjected for 19½ hours and the transfers from them showed no growth.

The total absence of growth in the transfers from the cultures subjected to fumigation and the abundant and vigorous growth in

the check cultures were constant and characteristic of this series of experiments.

Treatment with Coal Oil.

Early in the investigation of the problem of this mushroom disease it was learned that certain growers believed coal oil to be effective in destroying the spores of the fungus and checking the spread of the disease. The coal oil was poured on sections in the beds where diseased mushrooms had appeared and was also employed for the disinfection of the hands and tools. In order to demonstrate the efficacy or inefficacy of this treatment a large number of cultures of the fungus were grown on corn meal in 100-cubic-centimeter flasks and subjected to the direct action of the oil. These cultures were of various ages, but all in a state of vigorous growth. An arrangement was made by which the flasks could be inverted over the nozzle of a pipe supplying compressed air and made to pass through a stream of coal oil. The compressed air was turned on and the coal oil sprayed upon the culture. One half of the cultures were removed after the fungus had become covered with a film of coal oil. It was thought that this would be comparable to the condition in a mushroom house where coal oil was sprayed on the bed boards, walls, floors, and ceiling. The remaining cultures were sprayed until they were drenched with oil. In these the coal oil thoroughly penetrated the culture, which was practically an immersion of the fungus in coal oil.

As in the experiments with formaldehyde, transfers were immediately made from the treated cultures. These cultures grew as quickly and as vigorously as the check cultures (transfers made from the cultures before being treated with coal oil). A sufficient number of these experiments were made to demonstrate the inefficacy of coal oil as an agent for controlling this disease.

Treatment with Additional Disinfectants.

Costantin and Dufour (1893*a*) experimented with a variety of chemicals, to note their action on the growth of the fungus in cultures. The experiments were carried on in such a way that the toxic effect of the chemicals could be definitely determined. The following were used: Lysol, thymol (or thymic acid), boric acid, copper sulphate, calcium bisulphite, and milk of lime. These authors range the chemicals in the order of their effectiveness as follows: (1) Lysol (2 per cent solution), (2) thymol (2 per cent solution), (3) copper sulphate (2.5 per cent solution), (4) boric acid (to saturation). The milk of lime which many growers used for cleaning their caves was ineffective in preventing the growth of the disease, and the use of calcium bisulphite is not advised. From these experiments these authors advise the use of a 2 or 2.5 per cent solution of lysol as a spray to disinfect mushroom caves.

The investigators (Costantin and Dufour, 1892*b*, p. 145) described cultural experiments of the parasite, using sulphur dioxid produced by burning sulphur. It was concluded that "sulphur dioxid has a very destructive effect upon the spores of the parasite." Directions are given (Costantin and Dufour, 1893*b*, p. 411) for the fumigation of mushroom caves by burning sulphur. These investigators in a later publication say that the sulphur method of fumigation is attended with such inconvenience that spraying with lysol is preferable (Delacroix, 1900).

The Great Britain Board of Agriculture and Fisheries (1905) recommends the thorough spraying of the house or other structure in which the mushrooms are grown with a solution of sulphate of copper, 1 pound of sulphate to 15 gallons of water, three times at intervals of 10 days. This treatment has been recommended to English growers since 1905 (Gardeners' Chronicle, 1906–1912).

Costantin and Dufour (1893*a*, p. 510) found that copper sulphate had a very feeble antiseptic action on the parasite. In America the attempts to control the disease by this fungicide have been discouraging to the majority of the growers using it.

Costantin (1893*b*, p. 530) and Dufour (Costantin and Dufour, 1893*a*, p. 504), from the results obtained in experiments in a mushroom cave, advised the use of a 2.5 per cent solution of lysol. When the cave is dry, one spraying is said to be sufficient, but if it is very damp two thorough sprayings are to be given. A 2 per cent solution of lysol in water was used to check the spread of the disease in the mushroom bed in an infected cave. Places where the diseased mushrooms appeared in the beds were watered with the solution and the disease destroyed. In one of these places, watered with the disinfectant, a cluster of healthy, normal mushrooms later developed.

So-called "sanitary fluids," of which there are quite a number on the market, composed of coal-tar derivatives, saponified, are of a nature similar to the lysol used abroad.

Several experiments have been made with such fluids. Although the number of these experiments was limited and the results not absolutely conclusive, in view of the previous French experiments with a similar disinfectant it is thought that these sanitary fluids will be effective for the uses mentioned, while the price is not prohibitive.

PRACTICAL EXPERIMENTS TO CONTROL THE DISEASE.

A practical application was made of the information acquired from the results obtained in the laboratory experiments with formaldehyde gas in the inauguration of experiments for the control of the disease. This economic phase of the work received attention during several years. Continuous observations were made of the same houses during this period, but each succeeding year additional houses, with

their more or less differing conditions, were added as subjects of study in the practical application of the formaldehyde method for the control of the disease. Experiments were made in fumigating houses with different amounts of formaldehyde, the following proportions being used: 26 fluid ounces of formaldehyde per 1,000 cubic feet, 1 quart of formaldehyde per 1,000 cubic feet, and 3 pints of formaldehyde per 1,000 cubic feet. The proportion of 3 pints to 1,000 cubic feet was found to be more effective and will probably prove the most satisfactory in ordinary practice. In cases in which the leakage was considerable, allowance was made for such loss.

During the course of the present investigation 16 houses were fumigated by the writer or according to his directions. Two of these houses were fumigated at the rate of 26 ounces of formaldehyde per 1,000 cubic feet, five at the rate of 1 quart of formaldehyde per 1,000 cubic feet, and nine at the rate of 3 pints of formaldehyde per 1,000 cubic feet.

Two houses which were total failures the season previous to fumigation, after treatment produced crops which the grower reports as follows: "I believe that I never had a finer or more promising house or better mushrooms." The results of fumigation were successful in all cases in which the proper sanitary methods were observed to prevent reinfection of the houses.

From the writer's observations of the results of these experiments with fumigation and the satisfaction expressed by the growers in the course of conversation or correspondence as to the efficacy of the treatment, the important rôle of formaldehyde as an agent in controlling the mushroom disease seems practically demonstrated.

MEASURES OF CONTROL.

As a result of the present investigation of the Mycogone disease of mushrooms, the following measures may be advised for the control of the fungus. The treatment is more or less prophylactic in its nature and seeks rather to prevent the appearance or spread of the disease than to eradicate the fungus after it has actually made its appearance.

Sanitation Necessary in Relation to the Disease.

Too much emphasis can not be placed upon the danger from the fungus, because of its highly infectious nature. The remarkable rapidity with which the fungus is propagated and the great vitality possessed by the spores, as shown in the preceding pages, make it absolutely essential to observe great care in the construction of new beds or in passing from an infected to a noninfected bed. The ways in which the spores may be carried from place to place are numerous. They may be contained in the manure or soil for the casing of the beds, in particles of earth or manure adhering to the boots and shoes

of the workmen, or they may be present on tools and implements used in the mushroom houses. Wind and insects, especially the mushroom fly, are probably active agents in the distribution of the disease.

It is a deplorable fact that there are growers who allow diseased mushrooms to decay on the beds. There is in many cases so much discouragement due to losses occasioned by the disease that no effort is made to clean off the beds, the growers being content to pick what few normal mushrooms they can and avoid the labor necessary to suitably dispose of diseased specimens.

Figure 5 shows a photograph of a bed which has been practically exhausted, no normal mushrooms being produced. The grower has allowed the diseased masses to remain and decay and these will produce millions of spores, which will become a menace to new beds. These spores will become mixed with the manure and earth when the beds

FIG. 5.—Diseased mushrooms left to decay upon the beds to become a menace to future crops.

are removed and, if not suitably disposed of, may be introduced into the house when new beds are made.

All diseased material should be picked off as soon as it makes its appearance. The labor of keeping the beds clear of the diseased specimens will be repaid many times over in preventing the spread of the malady. An important measure for the control of the disease is to prevent the production of spores of the parasite.

Places in the beds where the fungus appears may be treated with either of the disinfectants mentioned, formalin or one of the "sanitary fluids," to prevent the spread of the disease. Diseased mushrooms picked from beds should be soaked with a disinfectant. For this purpose a solution of 1 gallon of formalin to 1 barrel of water (45 gallons) should be used. The only reason for not using formaldehyde solution is the discomfort in handling it. Its fungicidal action against the disease has proved to be effective. In place of the

formaldehyde solution, one containing 5 per cent—a gallon to a barrel of water—of one of the sanitary fluids composed of coal-tar derivatives will be satisfactory. This percentage is higher than that commercially recommended.

When the material is removed from the bed at the completion of the production of the crop, it should be immediately removed to places where there will be no possibility of bringing the disease back into the houses. The Mycogone disease, as far as is known, does not affect other cultivated crops. The old compost may therefore be used as a fertilizer, but it should be sold only to farmers who will carry it to a distance and to a locality where mushroom cultivation is not practiced.

The ground in the vicinity of the houses, the composting yard, and places with which the diseased materials have come in contact must be thoroughly sprayed with one of the disinfectants. If it is necessary to place the new manure or soil on ground where the old compost from diseased houses has rested, it will be necessary to give several such sprayings.

All tools, carts, wagons, and wheelbarrows which have been used to handle the infected materials must be thoroughly treated with the disinfectant.

Soil for the casing of the beds and for mixing with the manure must be selected from a place which has not been in contact with the disease.

The houses which have been fumigated should be kept closed until precautions to prevent the reentrance of the disease have been taken.

Directions for Fumigating Mushroom Houses.

Preparatory to fumigation, the houses should be completely cleaned of all old bedding material and thoroughly swept. The proper method for the disposal of this material has already been described.

A warm, moist day should be selected for fumigation, as the fungicidal effect of the gas is greater under such conditions. To this end, the house should be thoroughly sprayed with water and kept warm for about a week or ten days. To insure sufficient humidity, this process should be repeated the day before the fumigation is to be performed. The houses should be closed and sealed and made as nearly air-tight as possible by pasting paper over all cracks and filling up all openings, thus preventing the escape of the gas. If care is not exercised to prevent leakage of the gas, the fumigation may be rendered ineffective. The same grade of formaldehyde (or commercial formalin) as that used in the experiments with pure cultures of the fungus is advised for practical work.

Three pints of formalin should be allowed for every 1,000 cubic feet of space, the reagents being used in the proportion of 1 pint of formalin to one-half pound of potassium permanganate. In houses

24 by 100 feet, the usual size of mushroom houses, at least three receptacles should be used in which to generate the gas. It does not matter what the material of the receptacles may be, for the formalin has no corrosive action, but the heat of the chemical reaction, when potassium permanganate (permanganate of potash) is added to the formalin, might break glass receptacles. Half barrels, wash tubs, and iron or earthen receptacles are suitable, but it is advisable to select containers in which the diameter of the top is greater than that of the base. The formalin should cover the potassium permanganate when it is placed in the receptacle, which should be deep enough to insure the formalin from splashing over as a result of the vigorous chemical action.

The proper amount of formalin is measured and divided among the number of receptacles to be used in each house, while the proper proportion of potassium permanganate to be added to the formalin in each receptacle is carefully weighed out into paper or cloth bags. It will be found more satisfactory and the possibility of error may be avoided if like amounts of formalin and potassium permanganate are placed in each receptacle. If convenient, receptacles of a uniform size should be selected.

The weighing and measuring of the chemicals should be accomplished as quickly as possible after the receptacles containing the formalin are placed in the house. It is advisable to weigh the potassium permanganate into the bags first and then to measure the formalin for the respective receptacles, since considerable gas will be given off from the formalin and the house being almost air-tight, extreme physical discomfort due to the formaldehyde gas might result. The receptacles containing the formalin are then placed in position in the aisles of the house. In average-sized houses it will be sufficient to place the receptacles in the center aisle, but in larger houses the gas must be more evenly distributed by placing some of the receptacles in the several aisles. There will be required as many persons to place the bags of potassium permanganate in the formalin receptacles as the number of aisles in which the receptacles have been placed.

A bag of potassium permanganate is placed beside each receptacle containing the proportionate amount of formalin. When everything is ready, the operator in each aisle, if there are receptacles in more than one aisle, goes to the farthermost receptacle in that aisle and places the bag of potassium permanganate in the formalin, the operation being simultaneous in each aisle. Egress is made through the door at the end of the aisle, which is quickly closed and tightly sealed.

The operation should be accomplished as quickly as possible, and care should be taken to prevent accidents, for inhaling large quantities

of the gas may prove injurious. Formaldehyde has a distressing effect upon the eyes, and also attacks the mucous membranes, with consequent discomfort.

The potassium permanganate may be placed in the receptacles first, which should be of selected size, so that it will just cover the base of the receptacle. This was the method followed in the cultural experiments, but, as already stated, for general practice it was found more convenient to place the formaldehyde in the receptacles first.

The formalin-permanganate method of fumigation differs radically from other methods. The potassium permanganate is decomposed by a part of the formalin, and the heat of this chemical reaction serves to liberate formaldehyde gas.

Formaldehyde gas is *explosive* when in a confined place, such as a mushroom house; consequently, *all lights must be kept away from the houses while they are being fumigated*. Even after the receptacles containing the formalin are placed in the houses, they *should not be entered by persons with lights.*

The houses should be kept closed for at least 24 hours. If possible, they should be unopened until just before the new beds are to be installed, thus preventing any chance of their being infected meantime. Under no circumstance should the houses be opened until the manure which had been taken from them has been removed and the ground where it was placed thoroughly disinfected in the manner described.

CONCLUSIONS.

The disease of cultivated mushrooms is the cause of extensive losses to growers in this country, who state that unless precautions are taken to prevent its spread it will necessitate the abandonment of the industry in infected localities.

The disease of cultivated mushrooms apparently is the same as that which has caused great losses to foreign mushroom growers for many years.

This disease is caused by a fungus, a species of Mycogone, which has two forms of spores, one possessing thin and the other thick walls. Experiments prove that the thick-walled spores retain their vitality under ordinary cultural conditions for considerable periods of time. A moist atmosphere is essential for the growth of the fungus, as moisture rather than heat favors luxuriant growth. Cultures kept in a dry place were found to retain their vitality about 18 months. This would indicate that under natural conditions the life of the spores would be much longer.

The removal of the diseased mushrooms as soon as they appear will prevent the production of the thick-walled spores and thus lessen the spread of the disease.

As the disease is carried by many different means, the greatest care must be taken to prevent the infection of clean houses. There are, in general, two ways in which infection may take place: (1) It may be introduced into the house by means of the spawn and (2) the manure or soil for the beds may contain spores of the fungus. In the first case, the disease becomes evident as soon as the mushrooms begin to make their appearance, and all portions of the beds are affected. In the second case, beds may become infected by spores from a previously diseased crop. Air currents free these spores from crevices or wherever they may have lodged and thus assure a recurrence of the trouble; insects may carry spores from other diseased beds, or from diseased material which has been allowed to remain outside the houses, or spores may be carried on clothing or tools. When the spores of the parasite are introduced in such a manner, the disease may make its appearance a considerable time after the crop has begun to bear.

The abandonment of diseased houses for less than three years will be insufficient to rid them of the parasite, and a period of more than three years may be necessary.

Formaldehyde-gas fumigation and the observance of proper sanitary measures should be employed.

Formaldehyde gas, even in small quantities, retards the growth of the fungus, and when sufficiently strong will destroy the spores. A rate of 3 pints of formaldehyde or formalin per 1,000 cubic feet should be used, in the proportion of 1 pint of formalin to one-half pound of potassium permanganate.

Fumigation will control the disease in the houses, but will not keep them free, since bringing infected material, tools, etc., into the houses will certainly start the disease anew; therefore every precaution should be taken to prevent the reinfection of the houses after they have been fumigated.

Coal oil has no effect upon the spores of the parasite.

Diseased material should be removed from the houses immediately and treated with a disinfectant, preferably a solution of 1 gallon of formalin to about 45 gallons of water. This disinfectant should be used to spray all places where diseased material has been. Tools and conveyances should also be treated.

The disease is highly infectious, and the measures to be taken are more prophylactic than palliative in their nature.

Certain questions are yet to be solved concerning the life history of the fungus, such as the development of a perfect stage, but the method evolved for the control of the disease has proved effective and has resulted in saving large sums to mushroom growers.

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